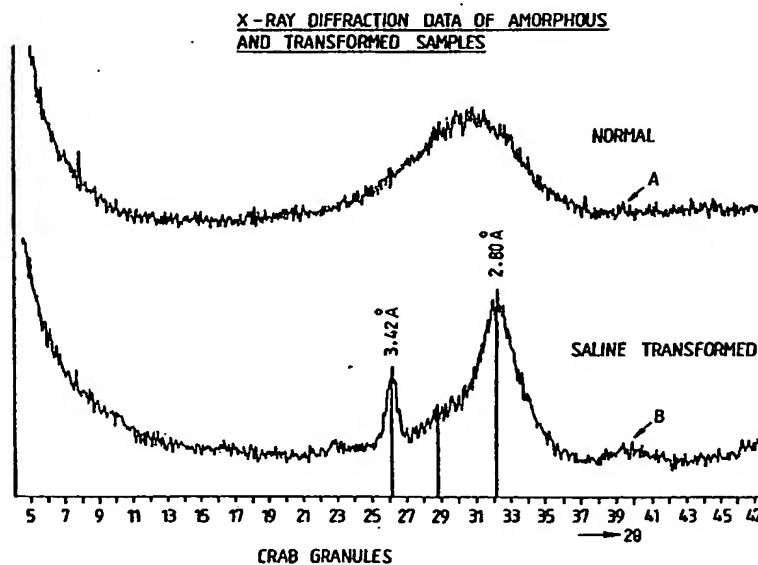




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C01B 25/32, A61L 27/00	A1	(11) International Publication Number: WO 94/02412 (43) International Publication Date: 3 February 1994 (03.02.94)
(21) International Application Number: PCT/GB93/01519 (22) International Filing Date: 20 July 1993 (20.07.93) (30) Priority data: 9215356.8 20 July 1992 (20.07.92) GB (71) Applicant (for all designated States except US): UNIVERSITY OF READING [GB/GB]; Industrial Liaison Bureau, Palmer Building, Whiteknights, Reading RG6 2AH (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : SIMKISS, Kenneth [GB/GB]; Foxhill Lane, Playhatch, Reading, Oxon RG4 9QF (GB). TAYLOR, Marina, Gloria [GB/GB]; 7 Valley Road, Henley-on-Thames, Oxon RG9 1RL (GB).	(74) Agent: DAVIES, Jonathan, Mark; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB). (81) Designated States: AU, BR, CA, JP, NO, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	

(54) Title: BONE MATERIALS



(57) Abstract

The invention provides a synthetic bone precursor material comprising amorphous calcium phosphate and a constituent that inhibits the crystallisation of calcium hydroxyapatite to amorphous calcium phosphate. The synthetic bone precursor may comprise a material of formula $\text{Ca}_x\text{Mg}_y\text{PO}_4$ wherein Mg acts as an inhibitor for crystallisation. Other crystallisation inhibitors such as pyrophosphate may be present. The inhibitor is capable of dissolving in physiological saline. Loss of inhibitor from the precursor allows crystallisation of hydroxyapatite. The invention relates to use of a synthetic bone precursor to form bone *in vivo*, by a transformation of the amorphous precursor material to crystalline hydroxyapatite bone material by the leaching action on inhibitory ions of body fluids. It is envisaged that the material will be useful in facilitating bone repair, inducing bone formation or assisting in the attachment of prostheses.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

BONE MATERIALS

The present invention relates to materials useful in producing bone mineral or similar material; to their preparation; and to their use.

Bone has organic and inorganic constituents. Most of the strength is due to the inorganic material, of which the major part is crystalline calcium phosphate, particularly hydroxyapatite, $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$. This crystalline material is strong, stable and essentially insoluble.

We have now found certain substantially amorphous materials that can be transformed under controlled conditions into crystalline bone material which may be of natural type or similar strong, stable material. Thus in one aspect the invention concerns use of certain precursor materials in the manufacture of a composition for use in a therapeutic technique in which bone is built up in vivo by transformation of the composition to produce bone material.

The precursor materials contain, as principal inorganic constituents, amorphous non-stoichiometric ~~compounds containing~~ calcium and phosphate ions. The phosphate ions may be mainly or wholly derived from phosphoric acid (H_3PO_4); they may include a proportion of ions derived from pyrophosphoric acid ($\text{H}_4\text{P}_2\text{O}_7$). Generally the compounds contain magnesium ions.

The precursor materials may be essentially inorganic, particularly when made synthetically. Alternatively they may include organic components. These can be deliberately included in synthetic materials, or be naturally present in naturally-derived materials.

Generally, the number of moles of magnesium per mole of calcium is in the range 0.001 to 1; for example 0.01 to 0.40 and preferably 0.1 to 0.16.

Material may be synthesised by bringing together calcium ions, magnesium ions, and phosphate ions in a buffer solution under conditions such that a material of desired composition is precipitated. Other components can be included in the reaction solution if desired.

Naturally-derived materials can be prepared from naturally-occurring deposits of amorphous calcium/magnesium/phosphate minerals. Suitable deposits occur in the soft tissue of many invertebrates (see. e.g. G.L. Becker *et al.*, J. Cell Biology, (1974), 61, 316-326 at page 322 and references cited therein), notably the intracellular granules from the digestive glands of various invertebrates, particularly crabs and snails e.g. as described in K. Simkiss *et al.*, J. Inorg. Biochem., (1990), 39, 17-23, and G.L. Becker *et al.*, *op.cit.* Thus as described by Simkiss *et al.*, granules can be extracted from C.maenas by homogenisation of the digestive gland and centrifugation in water to afford a clean white product. This product consists largely of inorganic phosphate, with the metal ions being mainly calcium but with some magnesium (the molar ratio being about 9: 1). The phosphate includes ions derived from phosphoric and pyrophosphoric acids, primarily from phosphoric acid. There are also organic components, mainly adenosine phosphates. (In general, such granule-derived material may contain significant amounts of nucleosides and glycoproteins.)

Materials may be prepared or selected which contain different proportions of one or more crystallisation inhibitor components such as magnesium and/or pyrophosphate and/or organic components, in order to control the rate of conversion to bone material under predetermined (generally physiological) conditions. At its simplest, a precursor material is an amorphous composition which contains calcium and phosphate ions as primary constituents together with inhibitor components (notably magnesium and/or pyrophosphate ions) which

inhibit its transformation to a crystalline form (generally based on hydroxyapatite). In use the precursor material gradually loses inhibitor components (in particularly, magnesium ions) by leaching action to ambient body fluids, and undergoes transformation into crystalline hydroxyapatite. Where pyrophosphate ions are used as crystallisation inhibitor the molar ratio of P_2O_5 to PO_4 is in the range 0.001 to 0.2, preferably 0.01 to 0.1. Other inhibitor components which could be used in the invention are citrate ions and/or acrylate ions.

In vivo, the precursor material is applied to a site where bone growth is required. The inhibitors will gradually be lost to body fluid, leading to gradual transformation to bone mineral.

Because the lixiviated mineral is a biologically compatible product it is unlikely to produce any allergic effects. Furthermore the transformation of amorphous material into bone mineral is slow, e.g. taking days, so that it is likely to integrate into normal healing processes. The bone mineral that is formed is believed to be compatible with collagen mineralisation which would make it particularly useful for assisting in bone repair. It is envisaged that the material will be useful in facilitating bone repair, inducing bone formation or assisting in the attachment of prostheses.

Some examples will now be described in more detail with reference to the accompanying drawing in which: Figure 1 shows X-ray diffraction data for amorphous material and crystalline material.

Examples

(1) Precursor materials: naturally derived

Material was isolated from *C.maenas* and characterised as described in Simkiss et al, op.cit, the content of which is incorporated herein by reference.

(2) Precursor materials: synthetic

Material was prepared by techniques adapted from Termine, J.D. et al, Arch. Biochem.Biophys.,

(1970), 140, 307-317 and 318-325, the content of which is incorporated herein by reference.

Calcium chloride solutions were made up in buffer (Tris HCl) to maintain the pH at about 7.4. Magnesium chloride was added to these solutions to produce a range of magnesium concentrations, the Mg: Ca mole ratio ranging from 1:1 to 0.01:1. A buffered phosphate solution (based on phosphoric acid) was added. The total concentrations were selected to approximate to 'natural' conditions: ion strength ca 0.15 (and pH ca 7.4). The solutions were stirred and the resulting precipitates were collected by centrifugation, followed by washing and drying at 60°C. The products were characterised by elemental analysis, IR spectroscopy and X-ray diffraction.

Transformation

Fig. 1 shows in trace A, an X-ray diffraction pattern of naturally-derived material prepared as specified at (1). A sample of the material was placed in physiological saline solution. The inhibitor ions will leach from the material. After many hours the sample was isolated, washed and dried and its X-ray diffraction pattern was determined again. This is trace B. It can be seen that there is clear evidence of ordering. That is, the material is no longer amorphous. The peaks representing d-spacings of crystalline hydroxyapatite (3.42Å and 2.80Å) are indicated.

Similar experiments were carried out with the synthetic samples prepared as specified at (2). It was found that the time taken to effect transformation varied with the concentration of magnesium in the sample. Samples in which some phosphate was substituted by pyrophosphate showed a similar variation in transformation time with pyrophosphate concentration.

The rationale behind this reaction probably involves the following:

- (i) Because the minerals are amorphous they are

more soluble than an equivalent crystal. They therefore start to dissolve.

(ii) As the minerals dissolve they tend to lose their high Mg content to the saline (in vivo, to the body fluids). This removes the inhibition to the nucleation of hydroxyapatite.

(iii) Bone mineral, which is less soluble than the amorphous deposit, now begins to form.

(iv) It is thought that initial bone deposits normally occur in gap regions of the organic collagen fibres. The crystals of bone in these collagen gaps are generally smaller than normal, probably because they contain magnesium ions. The bone crystals induced by the amorphous deposits may be of a similar type depending upon how fast the magnesium is lost.

(v) Because the bone mineral that has formed from these amorphous deposits is similar to normal bone it should integrate into the normal bone structures of the body and even be capable of being remodelled in vivo. In this it differs fundamentally from artificial adhesives.

It is possible to provide a wide range of materials based on amorphous $\text{Ca}_x\text{Mg}_y\text{PO}_4$, with different x:y ratios. These will have different solubilities and rates of transformation into bone mineral. This will influence their speed of cementing and the properties of the mineral induced. Thus it is possible to produce a series of simple amorphous materials which

(i) transform into bone mineral at predetermined rates (possibly with varying adhesion properties) when exposed to the body fluids

(ii) produce bone minerals of different physical properties (e.g. crystallinity)

(iii) integrate into normal bone structures

(iv) are capable of being remodelled in the body

and

(v) may act as a booster mechanism for normal bone repair.

Such substances are of value in providing an interface to assist the attachment of prostheses, promoting normal bone repair and replacing normal mineral in damaged bones or even teeth (and the term "bone material" as used herein is to be understood to cover synthetic tooth material).

The amorphous compositions of the invention could also be included in a matrix containing other materials: for example they could be integrated in a matrix with a biologically active moiety to provide an "active composite".

Furthermore, according to this invention it is possible to mix several amorphous calcium phosphate materials chosen to have different rates of transformation so as to provide for a series of transformations (either overlapping in time or sequential). Transformation time can be controlled by choice of inhibitor (i.e. one or several ions which may be the same or different for each amorphous calcium phosphate constituent of the mixture) and choice of inhibitor concentration and/or solubility.

An important aspect of the invention resides in the biomedical uses which may be made of the materials. A slow transformation with a varying range of the inhibiting ions (e.g. magnesium or pyrophosphate) encourages natural bone formation and repair mechanisms.

A mixture of fast-setting and slow-setting compositions of the invention will encourage vascularisation of an adhesive material such as a poly methyl methacrylate cement and make such mixtures suitable for coating the contact area of implanted bone prostheses.

When manipulated into pre-formed shapes, mixtures of fast and slow-setting materials may be used in spinal surgery as bone-grafting materials and bone filling materials for dealing with the collapse of intervertebral discs. No artificial material has yet been found

suitable for this application which has had to resort to the use of donor bone material from the pelvis of the same patient.

Claims

1. A synthetic bone precursor material comprising:
 - a) at least one amorphous calcium phosphate constituent, and;
 - b) at least one constituent which acts as an inhibitor for crystallisation of hydroxyapatite ($\text{Ca}_5(\text{OH})(\text{PO}_4)_3$) from amorphous calcium phosphate; which inhibitor is capable of dissolving in physiological saline and is capable of leaching from the precursor material to cause crystallisation of hydroxyapatite therefrom.
2. A synthetic bone precursor material according to claim 1 comprising an amorphous material of formula $\text{Ca}_x\text{Mg}_y\text{PO}_4$ wherein the molar ratio y to x is in the range of 0.001 to 0.4 and wherein Mg acts as inhibitor (b).
3. A material according to claim 1 or 2 wherein pyrophosphate (P_2O_7) is present in an amount such that the molar ratio of P_2O_7 to PO_4 is in the range of 0.001 to 0.2, and acts as inhibitor (b).
4. A material according to claims 2 and 3 containing both Mg and P_2O_7 inhibitors (b).
5. A material according to any preceding claim comprising a mixture of two or more different amorphous calcium phosphate constituents (a).
6. A method of making a material according to any of claims 1 to 4 which comprises bringing together calcium ions, phosphate ions and inhibitor ions in a buffered solution under conditions such that material of the desired composition is precipitated.
7. A method of making the material according to any of

claims 1 to 4 which comprises homogenisation, extraction and purification of a naturally-occurring deposit of amorphous calcium magnesium phosphate mineral.

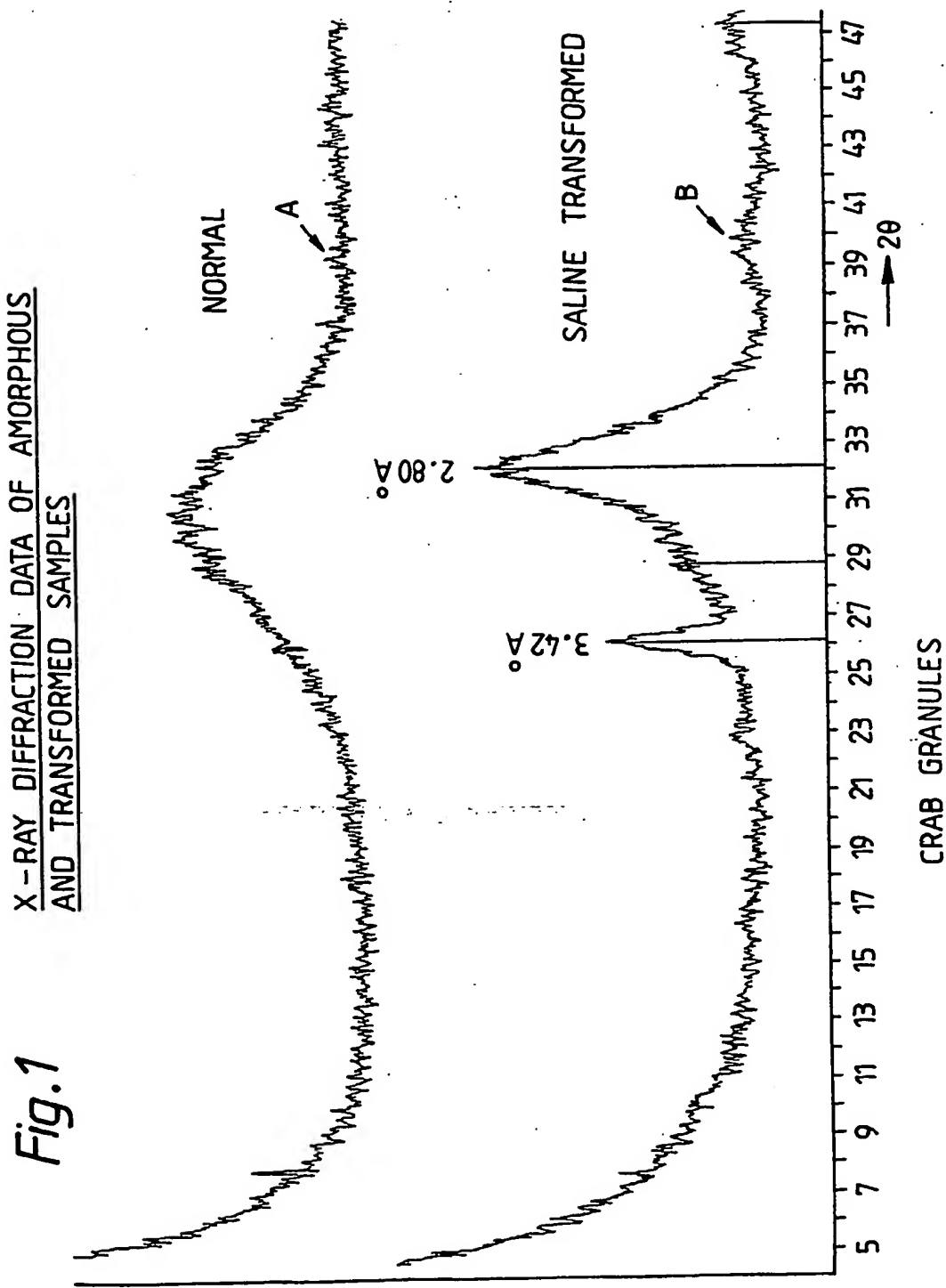
8. A synthetic bone precursor material according to any of claims 1 to 4 for use in the formation of bone material in vivo.

9. Use of a synthetic bone precursor material according to any of claims 1 to 4 in the manufacture of a composition for use in a therapeutic technique in which synthetic bone material is formed in vivo by transformation of the precursor amorphous material to crystalline bone material by the leaching action of ambient body fluids on inhibitor (b).

10. A method of treatment which results in the formation of synthetic bone material in vivo, wherein the method involves the application of a precursor material according to any one of claims 1 to 4 to the body of a human or animal patient.

11. A synthetic precursor bone material substantially as hereinbefore described, with reference to figure 1.

- 1 / 1 -



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

Intern. application No.

PC 93/01519

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C01B 25/32, A61L 27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61L, C01B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Dialog Information Services, File 351. World Patent Index 81-93, Dialog accession no. 009443464, WPI accession no. 93-136982/17, JGC CORP: "Artificial bone material - obtd. by using soln. mixer having outlets for hydroxy- -apatite precursor soln., alkali soln. and phosphoric acid soln. which are led to mixer to form prod." JP 5070113, A, 930323,9317 (Basic)	1,8-10
X	BE, A, 557314 (ETABLISSEMENTS HECTOR LEURQUIN), 7 November 1957 (07.11.57), claim 3	6
A	US, A, 4113500 (MASATOMI EBIHARA ET AL), 12 Sept 1978 (12.09.78), abstract	1-5,8-11

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

20 October 1993

2. 11. 93

Name and mailing address of the International Searching Authority

Authorized officer



European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

May Hallne

INTERNATIONAL SEARCH REPORT

International application No.

P GB 93/01519

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 8807498 (EWERS, ROLF), 6 October 1988 (06.10.88), abstract -----	7

INTERNATIONAL SEARCH REPORT
 Information on patent family members

26/08/93

International application No.

PCT/GB 93/01519

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
BE-A-	557314	07/11/57	NONE		
US-A-	4113500	12/09/78	DE-A,C-	2724972	08/12/77
			GB-A-	1548066	04/07/79
			JP-C-	1341726	14/10/86
			JP-A-	52147606	08/12/77
			JP-B-	60050743	09/11/85
			US-A-	4149894	17/04/79
WO-A1-	8807498	06/10/88	AU-B-	591839	14/12/89
			AU-A-	1570788	02/11/88
			CA-A-	1315694	06/04/93
			DE-A,C-	3709897	06/10/88
			EP-A,B-	0284074	28/09/88
			JP-T-	1502812	28/09/89
			US-A-	4938938	03/07/90